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Alternative splicing produces transcripts encoding two forms of the α subunit of GTP-binding protein G_o

(guanine nucleotide-binding protein/signal transduction)

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ABSTRACT **The α subunit of the guanine nucleotide-binding protein G_o ("o" for other) is believed to mediate signal transduction between a variety of receptors and effectors. cDNA clones encoding two forms of G_o α subunit were isolated from a mouse brain library. These two forms, which we call $G_oA\alpha$ and $G_oB\alpha$, appear to be the products of alternative splicing. $G_oA\alpha$ differs from $G_oB\alpha$ over the C-terminal third of the deduced protein sequence. Both forms are predicted to be substrates for ADP-ribosylation by pertussis toxin. $G_oA\alpha$ transcripts are present in a variety of tissues but are most abundant in brain. The $G_oB\alpha$ transcript is expressed at highest levels in brain and testis. It is possible that $G_oA\alpha$ and $G_oB\alpha$ have different functions.**

Guanine nucleotide-binding proteins (G proteins) form a large family of signal-transducing molecules. They are found as heterotrimers made up of α, β, and γ subunits. Members of the G protein family have been most extensively characterized by the nature of the α subunit, which binds guanine nucleotide, is capable of hydrolyzing GTP, and interacts with specific receptor and effector molecules (for reviews, see refs. 1–3). A variety of specific G protein α subunits and their corresponding cDNAs and genes have been identified. Some of the α subunits, such as stimulatory G protein (G_s) α subunit, are ubiquitous—i.e., splice variants of the G_s α subunit gene (4) are found in every tissue that has been examined. In reconstitution experiments, G_s α subunit was shown to interact with a specific subset of receptors and to activate adenylate cyclase. Other G proteins are cell-type specific—i.e., they are found in specialized cells and appear to transduce signals from only a small subset of receptors. For example, there are two transducins, one found only in rod photoreceptor cells and the other found only in cone cells (5, 6). Still other members of the α-subunit family are restricted to a subset of tissues.

In the course of the purification of inhibitory G protein (G_i) subunits, a new G protein was discovered and named G_o ("o" for other). The G_o heterotrimer is found abundantly in bovine brain as a membrane-associated protein (7, 8). Homologues of the G_o α subunit have been found in a variety of organisms from *Drosophila* to man (2, 9–12). In general, the G_o protein is localized in neural tissue and is an abundant membrane-bound protein in brain extracts. Evidence for the presence of G_o in some other tissues has also been obtained, but the highest concentration of both protein and mRNA appears to be in brain. The purified G_o α subunit has been used to reconstitute signal transducing systems, and it has been proposed to be the G protein that mediates a variety of processes that are sensitive to pertussis toxin inhibition (see ref. 13 for review). Perhaps the clearest evidence for the specific involvement of G_o comes from studies on ion channels in neuronal cells and heart atria. There is evidence from patch-clamp studies that the GTP-bound G_o α subunit can gate potassium and calcium channels (14–17). It also has been suggested that the pertussis toxin-sensitive activation of phospholipase C may be mediated by G_o α subunit (18, 19). Recently, the GAP 43 protein in nerve growth cones was shown to stimulate guanine 5′-[γ-thio]triphosphate binding to G_o (20). Thus, there are indications that a large variety of receptors and effectors could interact with the G_o α subunit.

The cDNA corresponding to G_o α subunit has been isolated and characterized from a number of different organisms (13). The gene itself is large, containing at least 10 exons that cover >90 kilobase pairs (kbp) of DNA in humans (21). During the course of studies to ascertain the diversity of the G protein α subunit family, we discovered cDNA clones encoding a variant form of mouse brain G_o α subunit. The sequences of the two forms are identical at the N terminus but show considerable variation at the C terminus*. These two forms of mouse brain G_o α subunit, which we call $G_oA\alpha$ and $G_oB\alpha$, are likely the products of alternative splicing, and they may have different functions.

MATERIALS AND METHODS

Polymerase Chain Reaction (PCR). PCR (22) was performed as described (23). Mouse brain and spermatid cDNA were made from poly(A)$^+$ RNA with random hexanucleotide primers by using Moloney murine leukemia virus reverse transcriptase. Conditions were those supplied by the manufacturer (BRL). The oligonucleotides used for PCR amplification of the cDNA were as follows: oMP19, CGGATCCAARTGGATHCAYTGYTT; oMP20, GGAATTCRTCYTTYTTRTTNAGRAA; oMP21, GGAATTCRTCYTTYTTRTTYAARAA; CT60, CATGCACGAATCCCTGAAGC; CT112, CCGCATGCACGAGTCTCTCAT; CT113, CCCGKAGRTTKTTGGCRATGA; CT114, ATGGGATGTACGCTGAGCGCA; GO1, TCGTCCTCGTGGAGCACCTG; and Ta29, GGGATCCNGTRTCNGTNGCRCANGT, in which R = A or G, Y = C or T, K = G or T, H = A, C, or T, and N = A, C, G, or T. PCR was performed on a Perkin–Elmer/Cetus thermal cycler. During each cycle, samples were denatured for 1.0 min at 94°C, extended for 1.0 min at 72°C, and annealed for 0.5 min at the following temperatures; oMP19 + oMP20 + oMP21, at 42°C; CT60 + Ta29, at 50°C; oMP19 + Ta29, at 42°C; GO1 + CT114, at 60°C; CT60 + CT113, at 55°C; and CT112 + CT113, at 55°C. Each oligonucleotide was used in the PCR at 10 ng/μl; 35 cycles were performed on approximately 5 ng of cDNA in a 50-μl reaction volume. The buffer and *Thermus aquaticus* (*Taq*) polymerase were supplied by Cetus.

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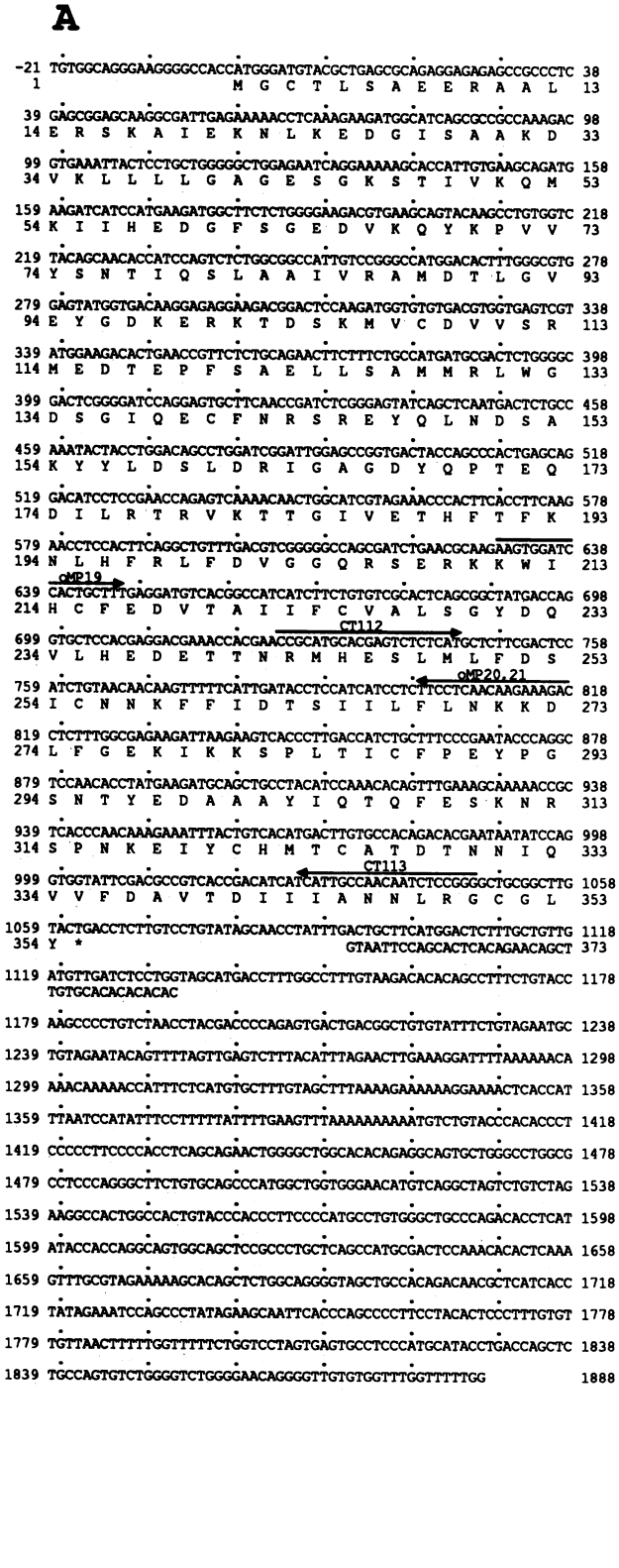
Abbreviations: PCR, polymerase chain reaction; G protein, guanine nucleotide-binding protein; G_i, G_s, and G_o, inhibitory, stimulatory, and "other" G proteins.

*The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M36777 for $G_oA\alpha$ and M36778 for $G_oB\alpha$).

Northern Analysis. Total RNA and poly(A)$^+$ RNA were run on 1% agarose gels and transferred to GeneScreen (DuPont) as described (24). A probe specific to both $G_oA\alpha$ and $G_oB\alpha$ was made by PCR amplifying clone G_o11 (Fig. 1*A*) with oligonucleotides GO1 and CT114 as described above. Probes specific to $G_oA\alpha$ or $G_oB\alpha$ were made by PCR amplifying G_o11 and 718-52-4 (Fig. 1*B*) with oligonucleotide pairs CT112 + CT113 ($G_oA\alpha$) and CT60 + CT113 ($G_oB\alpha$). The amplified products were run on low-melting-point agarose gels (Seaplaque, FMC), excised, and labeled by random priming as



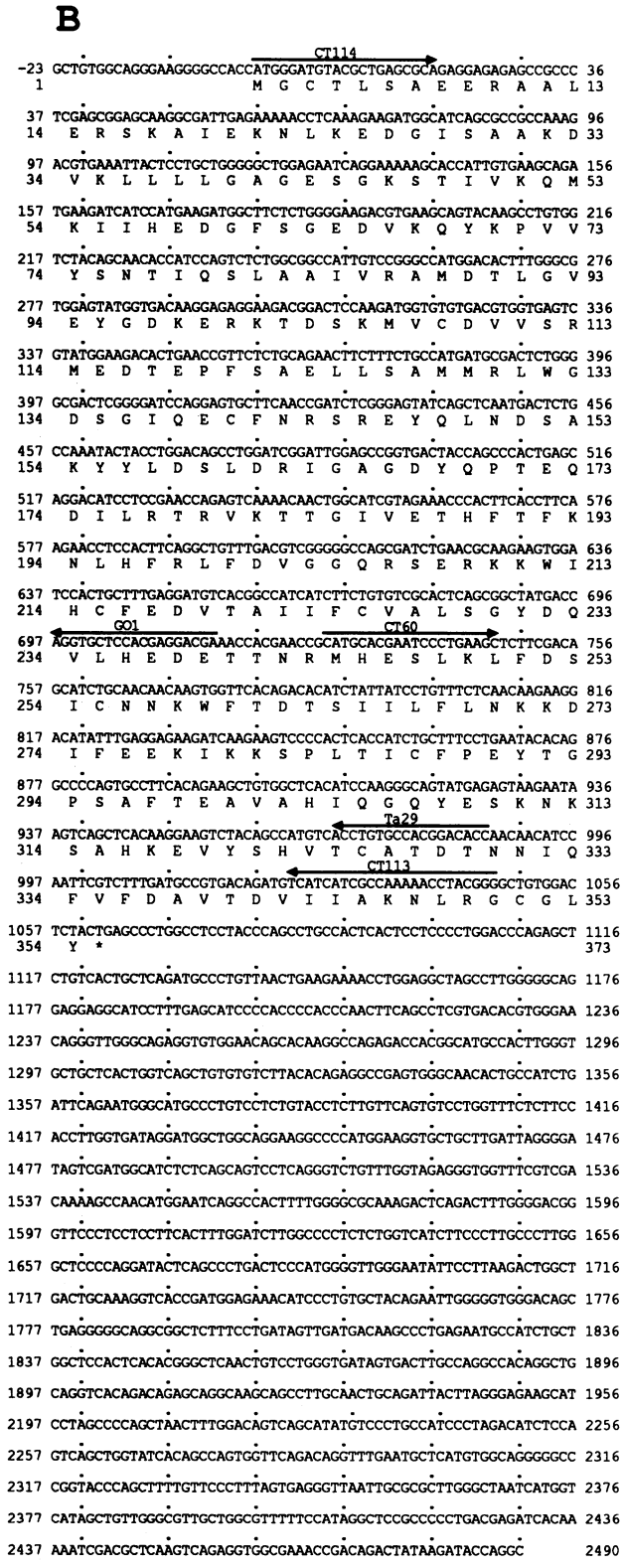


FIG. 1. Sequences of $G_oA\alpha$ (*A*) and $G_oB\alpha$ (*B*). (*A*) The sequence of $G_oA\alpha$ was obtained from two clones, G_o11 and G_o12. These clones were isolated from a random-primed mouse brain cDNA library. G_o11 extends from nucleotide −21 to 1133. G_o12 includes nucleotides 616–1888. G_o11 differs from G_o12 beginning at base 1093. These changes are shown below the G_o12 sequence. (*B*) The $G_oB\alpha$ sequence is contained in two clones, 718-52-4 and 718-52-5. These clones were isolated from the same library as the $G_oA\alpha$ clones. Clone 718-52-5 extends from nucleotide −23 to 903. Clone 718-52-4 extends from nucleotide 822 to 2490. Oligonucleotides mentioned in the text are indicated above the sequence.

described (23). The RNA blots were hybridized as described (23).

PCR Northern Analysis. PCR was performed on cDNA from total RNA as described above. The degenerate oligonucleotides oMP19, oMP20, and oMP21 were used for 30 cycles of amplification. The PCR products were electrophoresed through a 2% agarose gel, blotted to GeneScreen, and hybridized according to the manufacturer's instructions. These blots were probed with radiolabeled oligonucleotides specific to Gα11 (CTCGCTTAGTGCCACC), $G_oA\alpha$ (GAGCATGAGAGACTCG), and $G_oB\alpha$ (GAGCTTCAGGGATTCG). The oligonucleotides were end-labeled with [γ-^{32}P]-ATP as described (24). Blots were washed at room temperature twice for 5 min in 0.90 M NaCl/0.09 M sodium citrate (6× SSC)/0.1% SDS (24), twice for 5 min in 6× SSC, and finally for 1 min in 6× SSC at the melting temperature of each oligonucletoide.

Isolation of cDNAs and Nucleotide Sequencing. A random-hexanucleotide-primed mouse brain cDNA library in the cloning vector mλJSM8 (M.S. and M.I.S., unpublished data) was screened by standard techniques. Sequencing was performed by using the Sequenase kit marketed by United States Biochemical.

RESULTS

Cloning $G_oA\alpha$ and $G_oB\alpha$ cDNAs. PCR was used to screen RNA from both mouse brain and mouse spermatids for messages that correspond to different G protein α subunits (24). Degenerate oligonucleotides that code for amino acid sequences conserved in α subunits were used for amplification. These conserved amino acid sequences are shown in Fig. 1*A* (oMP19, oMP20, oMP21). The DNA sequences of clones derived from the PCR were determined. Two different classes of DNA sequences were found to encode amino acid sequences that correspond to the G_o α protein. Multiple clones of these two classes, termed PCRG-8 and PCRG-12, were obtained from both brain and spermatid cDNA. The derived amino acid sequence of PCRG-8 is identical to that found for the G_o α subunit cDNA from rat olfactory tissue (25). However, the sequence of PCRG-12 is identical to PCRG-8 only for the first 89 nucleotides and then diverges. To obtain a specific probe for the PCRG-12 gene sequence, we amplified mouse spermatid cDNA with oMP19 and another degenerate oligonucleotide, Ta29, which encodes conserved amino acids near the C terminus of α subunits (2). The PCR products were subcloned and screened with the PCRG-12-specific oligonucleotide, CT60 (Fig. 1*B*). The DNA sequence of one positive clone, 718-52, was found to be identical to PCRG-12, and it extended the sequence 168 nucleotides downstream. A hybridization probe was prepared from clone 718-52 by PCR with CT60 and Ta29 as primers. This probe and PCRG-8 were used to screen a randomly primed mouse brain cDNA library. Several cDNA clones were purified and sequenced. The complete DNA sequences of the mouse $G_oA\alpha$ cDNA and the mouse $G_oB\alpha$ cDNA are presented in Fig. 1. The amino acid sequence of mouse $G_oA\alpha$ differs from that of the rat G_o α subunit (25) at two positions (Thr-102 → Ala and Gly-166 → Ala). However, the DNA sequence of the $G_oB\alpha$ clone differs markedly from that of $G_oA\alpha$ in the region corresponding to the C-terminal third of the reading frame and the 3′ untranslated region. The DNA sequence is identical in the portion of the clone that corresponds to the N-terminal two-thirds of the protein and the 5′ untranslated region.

Another variant of the G_o α subunit cDNA clone was obtained whose sequence was identical to that of the $G_oA\alpha$ clones throughout the translated portion of the cDNA but varied from the $G_oA\alpha$ cDNA in the 3′ untranslated region of the sequence. The DNA sequence of the variable region is shown in Fig. 1*A*.

The nucleotide sequence of the $G_oA\alpha$ cDNA clone and that of the $G_oB\alpha$ cDNA clone are identical up to position 737. Thereafter some sequence conservation exists until a point corresponding to nucleotide 1064, which marks the end of the reading frame. In the 3′ untranslated region, the sequence of $G_oA\alpha$ and $G_oB\alpha$ differ dramatically from each other. It seems likely that $G_oA\alpha$ and $G_oB\alpha$ reflect different splice products derived from the same gene. Further support for this idea is provided by the intron–exon map of the G_o α subunit gene reported by Kaziro (21), which shows the same distribution of introns and exons as in the G_i α subunit gene. If the $G_oA\alpha$ and $G_oB\alpha$ gene products were the result of differential mRNA splicing, then we would expect the divergence point to be at nucleotide 723. This corresponds to an asparagine residue in both protein sequences. The first change in DNA sequence occurs at nucleotide 737 of the $G_oA\alpha$ sequence. On the basis of this interpretation, Fig. 2 shows the distribution of amino acid sequences corresponding to the nucleotide sequences of the $G_oA\alpha$ and $G_oB\alpha$ clones: after the point of divergence, there are 25 differences between the amino acid sequences, and 17 of them are concentrated within a stretch of 32 amino acids.

Distribution of G_o Gene Product. Tissue distribution of $G_oA\alpha$ and $G_oB\alpha$ mRNAs was analyzed first by Northern blots. Poly(A)$^+$ and total RNA from a variety of mouse tissues were separated by electrophoresis and hybridized to each of three probes that were obtained from the cDNA clones. These probes were made by PCR amplification of the 5′-end sequences shared by both $G_oA\alpha$ and $G_oB\alpha$ or by amplification of regions unique to either $G_oA\alpha$ or $G_oB\alpha$. Hybridization of the probe that is common to both splice products was compared with hybridization of the probes specific to $G_oA\alpha$ or $G_oB\alpha$ (Fig. 3). G_o α subunit mRNA was most abundant in brain (Fig. 3*A*), and on a longer exposure, six distinct transcripts were visible (Fig. 3*B*). These transcripts were also expressed at lower levels in testis, heart, and lung but were not observed in other tissues when assayed by Northern blot. The $G_oA\alpha$ probe specifically hybridized to the three smallest transcripts that were identified by the common probe. $G_oA\alpha$ was most abundant in brain but also was expressed in testis and heart (Fig. 3*C*). In contrast, $G_oB\alpha$ specifically hybridized to one transcript at equal intensity in brain and testis (Fig. 3*D*). On longer exposures, the largest transcripts could be seen to hybridize to both probes (data not shown) and may represent unspliced or partially spliced precursors derived from nuclear RNA. Thus, each transcript that hybridizes to the common probe is also recognized by either the GoAα- or GoBα-specific probe. The relative levels of $G_oA\alpha$ and $G_oB\alpha$ transcripts in a specific tissue can be estimated by comparing the relative intensities of the $G_oB\alpha$-specific message and the $G_oA\alpha$ transcripts (Fig. 3*B*). $G_oA\alpha$ was much more abundant than $G_oB\alpha$ in brain, whereas the levels of the two transcripts were similar in testis.

We used a technique based on PCR amplification that is more sensitive than Northern analysis (T.M.W., unpublished data) to get a clearer picture of the distribution and relative abundance of $G_oA\alpha$ and $G_oB\alpha$ mRNAs. cDNA from various tissues was amplified by PCR by using the degenerate oligonucleotides oMP19 in combination with oMP20 and oMP21. The amplified products were electrophoresed and stained with ethidium bromide. All tissues had an amplified band of the same size, indicating that they all express G protein α subunit mRNA. A second assay (Fig. 4) also was used to demonstrate that each tissue supported the PCR reaction. Southern blots of the PCR products were hybridized with an oligonucletoide probe that is specific to the G protein α subunit designated Gα11. This protein is expressed ubiquitously (24). All tissues expressed roughly the same amount of

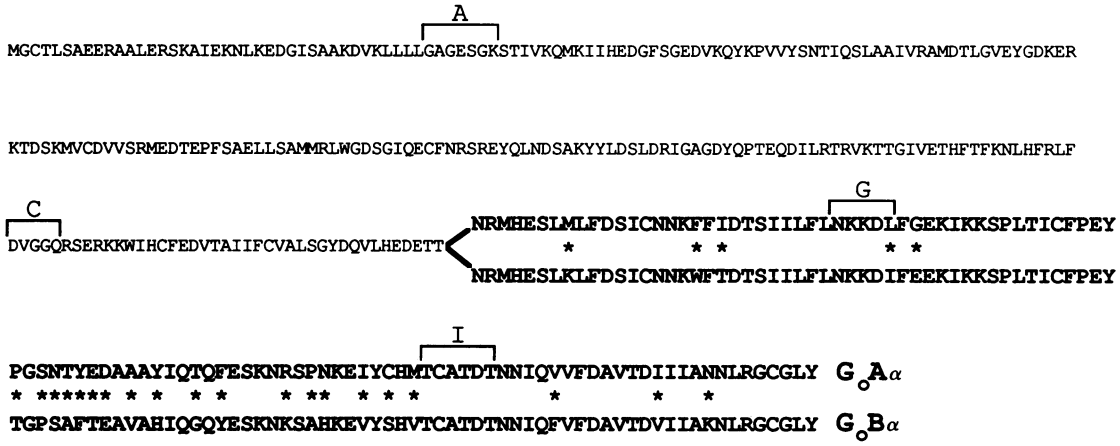


FIG. 2. Comparison of $G_oA\alpha$ and $G_oB\alpha$ amino acid sequences. Differences in amino acid residues are indicated by stars between the two sequences. A, C, G, and I represent domains that have been implicated in guanine nucleotide interactions (2).

$G\alpha 11$ with the exception of uterus, which might reflect a lower level of $G\alpha 11$ in this RNA source. Southern blots of the PCR products were also hybridized with oligonucletoide probes specific to either $G_oA\alpha$ or $G_oB\alpha$ (Fig. 4). $G_oA\alpha$ was most abundant in brain, followed by testis, heart, skeletal muscle, and uterus. $G_oA\alpha$ was also expressed at low levels in intestine, kidney, and lung and at exceedingly low levels in spleen and thymus; it was undetectable in liver. These cDNA samples were also amplified by PCR with oligonucleotide primers specific to $G_oA\alpha$. The results showed the same relative distribution pattern of $G_oA\alpha$ transcripts in the different tissues (data not shown). In contrast, $G_oB\alpha$ was expressed primarily in brain and testis and to a lesser extent in lung, but other tissues expressed little or no $G_oB\alpha$ mRNA. The expression pattern of $G_oA\alpha$ and $G_oB\alpha$ was corroborated by PCR analysis using a second set of independently isolated RNA samples. The relative levels of either $G_oA\alpha$ or $G_oB\alpha$ expressed in brain and testis and assayed by PCR agreed with the Northern analysis.

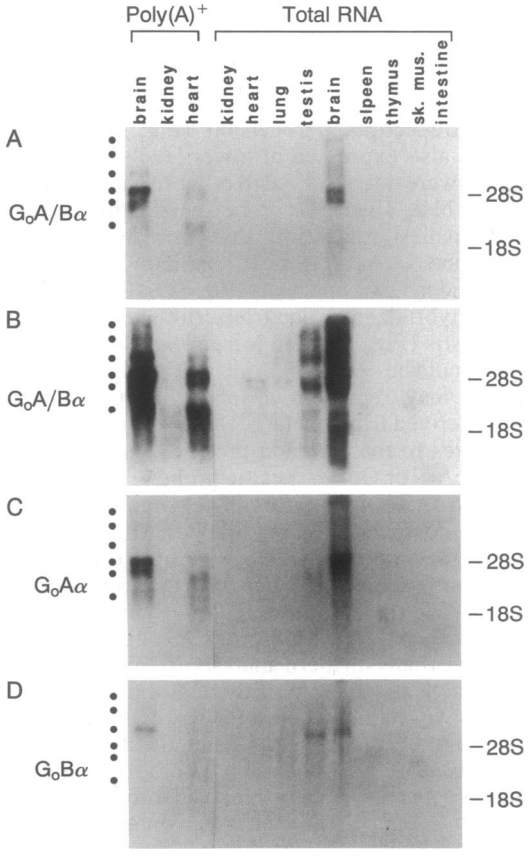


FIG. 3. Northern analysis of $G_oA\alpha$ and $G_oB\alpha$. Probes specific to both $G_oA\alpha$ and $G_oB\alpha$ [$G_o(A/B)\alpha$] (*A* and *B*), to $G_oA\alpha$ (*C*), and to $G_oB\alpha$ (*D*) were hybridized to poly(A)$^+$ and total RNA from various mouse tissues. Total RNA (20 μg) was loaded per lane. The poly(A)$^+$ RNA was loaded as follows: brain, 0.5 μg; kidney, 5 μg; and heart, 5 μg. *B* is a longer exposure of *A*.

DISCUSSION

The G_o α subunit gene is a complex locus including more than 10 exons and extending over 90 kbp of DNA in humans (21). We have demonstrated the existence in mouse of transcripts that code for two forms of the G_o α protein, $G_oA\alpha$ and $G_oB\alpha$. The sequences of these transcripts are identical for over 700 nucleotides at their 5′ ends. They diverge near an intron/exon junction, suggesting that they are products of alternative splicing. Kaziro and coworkers have now confirmed this interpretation by determining the organization of exons that represent both forms (Y. Kaziro, personal communication).

Heterogeneity of proteins antigenically related to the G_o α subunit has been observed. Goldsmith *et al.* (27) resolved on two-dimensional gels $G_oA\alpha$ and another protein from bovine brain, termed G_o^*, that reacted with four G_o α subunit-specific antisera. However, only one of the peptide antisera used in this study was raised against a sequence not shared by $G_oA\alpha$ and $G_oB\alpha$. This sequence, the carboxyl-terminal decapeptide of $G_oA\alpha$, differs from that of $G_oB\alpha$ at only one residue. Consequently, this antisera may recognize $G_oB\alpha$. In addition, G_o^* can be ADP-ribosylated by pertussis toxin. $G_oB\alpha$ is

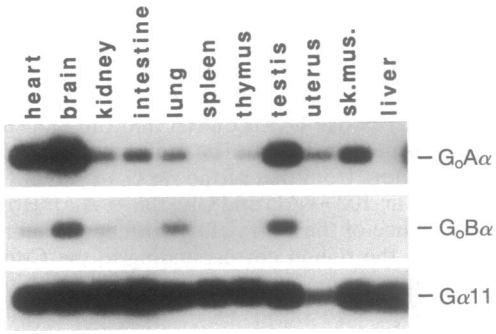


FIG. 4. PCR Northern analysis. PCR was performed on various mouse tissues with the degenerate oligonucleotides of oMP19, oMP20, and oMP21 (Fig. 1). The amplified products were hybridized with radiolabeled oligonucleotides specific to $G_oA\alpha$, $G_oB\alpha$, and $G\alpha 11$.

predicted to be a substrate for pertussis toxin because the fourth residue from the C terminus is cysteine. A cysteine residue at this position is implicated as the site of ADP-ribosylation in all α subunits that are sensitive to pertussis toxin (1, 2). Thus, it is possible that G_o^* is the $G_oB\alpha$ protein.

Milligan *et al.* (28) identified, in rat myometrical membranes, a protein that reacted with two G_o α subunit-specific peptide antisera but not with an antiserum against partially purified G_o α subunit or with a peptide antiserum against $G_oA\alpha$ amino acids 22–35. Although we do not have sequence data to suggest differential splicing at the N terminus of G_o α subunit, it is interesting that the N-terminal 39 amino acids of the *Drosophila* homolog are switched by an alternative splicing mechanism (9–11).

The 3′ untranslated region of $G_oA\alpha$ also appears to undergo alternative splicing. We sequenced two clones that diverge 29 nucleotides past the stop codon (Fig. 1*A*). Murtagh *et al.* (29) found two forms of the $G_oA\alpha$ message in bovine retina that diverge 31 nucleotides past the stop codon. The significance of these alternately spliced 3′ untranslated regions is unknown. However, the untranslated regions of specific G protein α subunit isotypes are highly conserved across species (13, 30), revealing a selective pressure to maintain these sequences. Hence, the untranslated regions may affect gene regulation at the transcriptional or posttranscriptional level (31).

The functional significance of two forms of G_o α subunit is not clear. Masters *et al.* (32) have shown that the C-terminal 40% of $G_s\alpha$ subunit contains the structural elements required for specific interactions with effector and receptor. Perhaps $G_oA\alpha$ and $G_oB\alpha$ interact with different sets of receptors and/or effectors. Indeed, amino acids 315–327, by analogy to transducin, are important for receptor interactions (33). Within this region, $G_oA\alpha$ and $G_oB\alpha$ differ at 5 positions. Between amino acids 292 and 307, the two sequences differ at 11 positions. However, the contribution of this region to G protein function is not defined.

The list of genes known to encode G proteins is growing. Many novel α (24), β (ref. 34; E. von Weizsäcker and M.I.S., unpublished data), and γ (35) subunit sequences have been cloned recently. Alternative splicing provides another means to increase G protein diversity and thereby to expand the variety of G protein-mediated signaling events in a multicellular organism.

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